Performance Monitoring Protocol (QA/QC) for the Agilent Headspace GC/MS

1 Scope

This document addresses the performance monitoring (QA/QC) of the Agilent GC/MS System with a headspace autosampler, which may include optional detectors, such as a Flame Ionization Detector (FID) or Nitrogen Phosphorus Detector (NPD). This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA, in the following disciplines (sub-disciplines): Seized Drugs, Toxicology, Fire Debris and Explosives (Fire Debris, Explosives Chemistry), and Materials (Trace) (General Chemistry).

2 Principle

The Agilent Headspace GC/MS is a gas chromatograph (GC) with a headspace autosampler. The system may also be equipped with additional detectors, such as FID and NPD. A headspace autosampler is a device used to sample the gas phase volatile analytes within a sealed vial. This sampling is transferred to the inlet of the GC and onto a column where the components are separated and sent to the detector. There may be two columns in the Agilent GC (labeled front and back), each leading to respective detectors. The front column is a capillary column which leads to a single quadrupole Mass Selective Detector (MSD) Mass Spectrometer. The MSD is configured with a dedicated electron impact ionization (EI) source in the mass spectrometer, and is referred to as the HS-MSD throughout this document. The back column leads to a FID or NPD depending on which instrument is used. This portion of the instrument is referred to as the HS-FID or HS-NPD throughout this document. The headspace autosampler can be configured to inject into either inlet.

This performance monitoring protocol is based upon the manufacturer's recommendations. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

a. Instrumentation - Agilent 7890 Gas Chromatograph, Agilent 5975 or 5977 MSD with EI Source, FID (if equipped), NPD (if equipped), Chemstation Software, MassHunter Software, Gerstel Master, and/or Gerstel Maestro Software (or equivalent)

b. Autosampler - Gerstel automated sampler, accessories, and software (or equivalent)

c. Capillary GC Columns:

Agilent DB-624, 30 m, 0.25 mm i.d., 1.4 µm film (or equivalent) (MSD)
Restek RT-QS-Bond 30 m, 0.32 mm i.d., 10 µm film (or equivalent) (NPD)
Restek RTX-BAC-2, 30 m, 0.32 mm i.d., 1.2 µm film (or equivalent) (FID)

d. Carrier Gases:
Helium, 99.99% (high purity) (MSD, FID)
Nitrogen, 99.99% (high purity) (NPD)

e. Perfluorotributylamine (PFTBA, FC-43) (Agilent or equivalent)

f. Hydrogen gas (high purity)

g. Compressed air

h. Ethanol (200 Proof)

i. Isopropanol (HPLC Grade)

j. Chloroform (HPLC Grade)

k. Methanol (HPLC Grade)

l. Methy Ethyl Ketone (HPLC Grade)

m. Nitromethane (HPLC Grade)

n. Acetone (HPLC Grade)

o. Toluene (HPLC Grade)

p. Deionized Water, 18 MΩ·cm Milli-Q or equivalent

q. Potassium cyanide (Reagent Grade)

r. Sodium hydroxide (Reagent Grade)

s. ~5 N (20% w/v) Sodium Hydroxide (NaOH):
To a 100-mL beaker or Erlenmeyer flask, add 60 mL water and 20 g sodium hydroxide. Mix well to dissolve and bring to volume with deionized water. Store in a Nalgene container at room temperature. Stable for 1 year.

t. Acetonitrile (HPLC Grade)

u. 5 N Sulfuric Acid (H_2SO_4) (Reagent Grade)

v. Autosampler vials - 10 or 20 mL crimp-top headspace autosampler vials or appropriate headspace vials for Gerstel autosamplers (or equivalent)

w. Injection port septa - standard low-bleed 11 mm (Agilent or equivalent)

x. Injection port liners - 4 mm split-splitless, tapered, with or without glass wool (Agilent or equivalent)

y. Autosampler syringes - 2.5 mL headspace and 1 mL liquid syringes (Gerstel or equivalent)

4 Standards and Controls

The testmix is used to assess daily operating performance and continued integrity of the system. It will be analyzed and evaluated prior to the analysis of evidence.

4.1 Testmix (Toxicology/General Chemistry) for HS-MSD

Refer to the analyte-specific SOP for unique samples (e.g., GHB, cyanide) for the preparation of the positive control, which will be used as the testmix.

For general volatiles analysis, prepare the testmix by adding 500 mL of deionized water into a 1000-mL volumetric flask. Add 0.1 mL each of ethanol and isopropanol. Add 0.01 mL of chloroform. Bring to the mark with deionized water. Store refrigerated in glass or plastic. Stable for at least one year, verified with each use.

Transfer 0.5 mL of the solution into a 10-mL headspace vial. Alternatively, transfer 1.0 mL of the solution into a 20-mL headspace vial.

4.2 Testmix (Explosives Chemistry, Fire Debris) for HS-MSD

Prepare the testmix by adding 50 mL of deionized water into a 100-mL volumetric flask. Add 0.01 mL each of methanol, ethanol, isopropanol, methyl ethyl ketone, nitromethane, acetone, and toluene. Bring to the mark with deionized water. Store refrigerated in a tightly sealed container. Stable for two years. This preparation may be appropriately scaled.

Refer to the analyte-specific SOP for unique samples, such as TATP, for the preparation of the positive control, which will be used as the testmix.

4.3 Testmix for HS-NPD

a. Cyanide Stock Standard (0.2 mg/mL):
Prepared by adding 50 mg of potassium cyanide to a 100-mL volumetric flask

containing 2 mL of ~5 N NaOH. Dilute to volume with deionized water and mix thoroughly. Store at room temperature in a tightly sealed glass or plastic container. Stable for at least 1 year, verified with each use.

b. 0.08% Acetonitrile (v/v) (Internal Standard):
Add 80 µL acetonitrile to about 90 mL deionized water in a 100-mL volumetric flask. Dilute to volume with deionized water and mix thoroughly. Store at room temperature in a tightly sealed glass or plastic container. Stable for at least one year, verified with each use.

c. Aqueous Positive Control (10 µg/mL cyanide):
Prepared by adding 5 mL of the Cyanide Stock Standard into to a 100-mL volumetric flask. Dilute to volume with deionized water and mix thoroughly. Stable for at least one year, verified with each use.

d. Performance Verification Sample:
Measure 0.5 mL of the Aqueous Positive Control (10 µg/mL cyanide) and 25 µL of 0.08% acetonitrile (Internal Standard) into a 10-mL headspace vial and cap. Using a 2.5 cc syringe, inject 0.5 mL of 5 N H_2SO_4 into the vial and thoroughly vortex the sample to uniformly distribute the acid. Wipe any residual H_2SO_4 from the septum and/or cap. Allow the sample to equilibrate at room temperature for 30 minutes.

4.4 Testmix for HS-FID

Volatiles Testmix Solution (0.01 % v/v):

Add 500 mL deionized water to a 1000-mL volumetric flask. Add 0.1 mL each methanol, ethanol, isopropanol, and acetone. Bring to the mark with deionized water and mix well. Store refrigerated in glass or plastic. Stable for at least one year, verified with each use.

Transfer 0.5 mL of the testmix solution to a 10 mL headspace vial. Alternatively, transfer 1.0 mL of the testmix solution to a 20 mL headspace vial. Stable for at least one year, verified with each use.

4.5 PFTBA Tuning Solution for HS-MSD

The PFTBA tuning solution is used for tuning the mass spectrometer and verifying mass calibration. It is supplied by the instrument manufacturer and does not expire. It is stored in a glass container attached to the MSD. Under normal conditions, this should not need to be refilled.

5 Sampling

Not applicable.

6 Procedure

6.1 Daily Checks – HS-MSD

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

a. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.

b. Record the line pressure of the building helium supply (carrier gas). The regulator should read 50 p.s.i. or above. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the helium is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.

c. Check the Ion Gauge to ensure that there are no significant leaks in the system. Do not use if the pressure is higher than 6×10^{-5} torr.

d. Perform a tune of the instrument. If Autotune (ATUNE) is selected, the mass spectrometer will tune itself using PFTBA. Evaluate the results using the 'Decision Criteria' in section 8.1. If the results are acceptable, save and print the tune file (ATUNE) when completed.

e. Refer to the analyte-specific SOP for unique samples (e.g., GHB, cyanide, TATP) for the appropriate procedure, instrumental conditions, and decision criteria for performing an analysis of the testmix. For general volatiles analysis perform a HS-MSD analysis of the applicable testmix (section 4.1 or 4.2) prior to the analysis of evidence. Open the appropriate testmix instrument method, and verify the parameters as listed in section 7.1 or 7.2. Analyze the appropriate testmix and evaluate the results using the 'Decision Criteria' in section 8.2.

f. If all requirements are within specification, print the TIC and representative mass spectra. If any requirements fail, contact appropriate instrument support personnel.

6.2 Daily Checks – HS-NPD

The following steps will be performed daily. Enter the appropriate information in the QA/QC log.

a. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.

b. Record the line pressure of the building nitrogen supply (carrier gas). The regulator should read 50 p.s.i. or above. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the instrument is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. is remaining.

c. Ensure that the NPD is operational using the front panel controls of the GC.

d. Ensure that the autosampler injects into the appropriate GC inlet.

e. Prepare a Performance Verification Sample as directed in section 4.3.d and analyze using the parameters provided in section 7.3. Alternatively, refer to the analyte-specific SOP for unique samples (e.g., azide) for the appropriate procedure, instrumental conditions, and decision criteria for performing an analysis of an applicable positive control.

f. Evaluate the results using the 'Decision Criteria' in section 8.3. If all requirements are within specification, print the chromatogram(s). If any requirements fail, contact appropriate instrument support personnel.

6.3 Daily Checks- HS-FID

The following steps will be performed daily. Enter the appropriate information in the QA/QC log.

a. Record the remaining disk space on the hard drive. Use Windows Explorer program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.

b. Record the line pressure of the building nitrogen supply (carrier gas). The regulator should read 50 p.s.i. or above. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the instrument is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. is remaining.

c. Ensure that the FID flame is lit.

d. Ensure that the autosampler injects into the appropriate GC inlet.

e. Analyze the HS-FID Volatiles Testmix using the parameters provided in section 7.4.

f. Evaluate the results using the 'Decision Criteria' in section 8.4. If all requirements are within specification, print the chromatogram(s). If any requirements fail, contact appropriate instrument support personnel.

6.4 As Needed Checks

The following steps will be performed as needed based on system performance. Enter the appropriate information in the QA/QC log to indicate completion.

a. Replace the septum in the GC injection port.

b. Replace the liner within the GC injection port.

c. Check the GC syringe in the autosampler. Replace if needed.

d. Check the internal bungee cords in the autosampler (if equipped). Replace if needed.

e. Check the plungers in each autosampler syringe. Replace if needed.

7 Instrumental Conditions

7.1 HS-MSD (Toxicology/General Chemistry) Testmix Parameters

7.1.1 Headspace Sampler Parameters

Incubation temperature:	80°C
Incubation time:	10 min
Agitator speed:	300 RPM
Agitation timing:	10 sec on 1 sec off
Syringe temperature:	90ºC
Sample fill volume:	1.0 mL
Sample fill rate:	1.0 mL/sec
Sample fill strokes:	5
Sample injection speed:	1.0 mL/sec
Syringe flush time:	1.0 min

7.1.2 Gas Chromatograph Parameters

<u>Oven</u>

Temperature:	50ºC for 3 min
Ramp:	10ºC/min to 250ºC for 5 min
Run time:	28 min
Equilibration time:	0 min

Column

Type:	DB-624
Length:	30 m
Internal diameter:	0.25 mm
Film thickness:	1.4 µm

Inlet/Carrier

Inlet temperature:	150ºC
Injection mode:	Split
Carrier gas:	Helium, 99.99% (split)
Carrier mode:	Constant flow
Pressure:	6.5 psi
Split ratio:	10:1

7.1.3 Mass Spectrometer Parameters

Ionization mode:	Electron impact
Scan mode:	Full scan
Scan range:	27 – 400 m/z
Relative voltage:	106 V
Source temperature:	230ºC
Transfer line temperature:	260ºC
Quadrupole temperature:	150ºC
Solvent delay:	1.75 min

7.2 HS-MSD (Explosives Chemistry, Fire Debris) Testmix Parameters

7.2.1 Headspace Sampler Parameters

Incubation temperature:	80°C
Incubation time:	5 min
Agitator speed:	300 RPM
Agitation timing:	10 sec on 1 sec off
Syringe temperature:	90ºC
Sample fill volume:	1.0 mL
Sample fill rate:	1.0 mL/sec
Sample fill strokes:	5
Sample injection speed:	1.0 mL/sec
Syringe flush time:	4.0 min

7.2.2 Gas Chromatograph Parameters

<u>Oven</u>

Temperature:	40ºC for 4 min
Ramp:	10ºC/min to 120ºC for 0 min
Run time:	12 min
Equilibration time:	0.25 min

<u>Column</u>

Type:	DB-624
Length:	30 m
Internal diameter:	0.25 mm
Film thickness:	1.4 µm

<u>Inlet/Carrier</u>

Inlet temperature:	150ºC
Injection mode:	Split
Carrier gas:	Helium, 99.99% (split)
Carrier mode:	Constant pressure
Pressure:	5.3 psi
Split ratio:	10:1

7.2.3 Mass Spectrometer Parameters

Ionization mode:	Electron impact
Scan mode:	Full scan
Scan range:	29 – 400 m/z
Relative voltage:	106 V
Source temperature:	230ºC
Transfer line temperature:	260ºC
Quadrupole temperature:	150ºC
Solvent delay:	2.0 min

7.3 HS-NPD Testmix Parameters

7.3.1 Headspace Sampler Parameters (NPD)

Syringe:	2.5 mL-HS
Oven / syringe temp.:	45°C / 55°C
Flush time:	4.0 min
Incubation time:	5.0 min
Agitator speed:	250 rpm
Agitation timing:	10 sec on 1 sec off

Injection volume:	250 µL
Fill speed / strokes:	500 µL/sec / 5
Incubation time:	5.0 min
Injection speed:	1000 µL/sec
Injection penetration:	40 mm

7.3.2 Gas Chromatograph Parameters (NPD)

Oven

Temperature:	110ºC for 0 min
Ramp:	4ºC/min to 130ºC for 5 min
Run time:	10 min
Equilibration time:	0.2 min

Column

Type:	RT-QS-Bond
Length:	30 m
Internal diameter:	0.32 mm
Film thickness:	10 µm

Inlet and Carrier

Inlet temperature:	150°C
Injection mode:	Purged
Carrier gas:	Nitrogen
Carrier mode:	Constant flow

7.3.3 Detector Parameters (NPD)

NPD

Temp.:	225°C
Offset:	20
Equilibration:	0.01 min
Air flow:	60 mL/min
Hydrogen flow:	3.0 mL/min
Electrometer:	ON

7.4 HS-FID Testmix Parameters

7.4.1 Headspace Sampler Parameters (FID)

Incubation temp.:	60°C
Incubation time:	30 min
Agitator speed:	250 rpm
Agitation timing:	10 sec on, 1 sec off

Syringe:	2.5 mL-HS
Syringe temp.:	70°C
Sample fill volume:	500 µL
Sample fill rate:	500 µL/sec
Sample fill strokes:	5
Sample injection rate:	500 µL/sec
Syringe flush time:	2.0 min

7.4.2 Gas Chromatograph Parameters (FID)

<u>Oven</u>

Temp.:	40°C
Isothermal run time:	6 min
Equilibration time:	0.2 min

<u>Column</u>

Type:	RTX BAC-2
Length:	30 m
Internal diameter:	0.32 mm
Film thickness:	1.2 µm

<u>Inlet and Carrier</u>

Inlet temp.:	200°C
Injection mode:	Split
Carrier gas:	Helium
Carrier mode:	Constant pressure
Pressure:	10.2 psi
Split ratio:	1:1

8 Decision Criteria

8.1 MSD Tune

Verify the results of the tune. Compare the results of the tune to previous tune results. Significant voltage increases or changes in the isotope ratios indicate the need to initiate corrective maintenance procedures. The following are typical ATUNE values for the MSD:

a. PFTBA Tune: Mass ±0.4 for m/z 69, 219, and 502

b. Peak width: 0.45-0.65

c. Relative abundance: 69 greater than 50%
219 greater than 50%
502 greater than 1%

8.2 HS-MSD Testmix

Verify the results of the testmix.

a. In order for the instrument to be considered in good operating condition, all testmix components should generate well-resolved, Gaussian-shaped peaks with baseline separation.

b. A SNR of 3:1 will be the minimum response necessary to consider a response a peak.

c. There should be no significant extraneous peaks in the chromatogram.

d. The retention times of each component should be similar to previous analyses (unless GC maintenance has been performed, such as column clipping or replacement).

e. Check for the correct mass assignments for the mass spectra. In order for the MS to be considered in good operating condition, the correct mass assignments for each of the analytes in the appropriate testmix should be present. The following ions at m/z should be present:

Toxicology/General Chemistry
- ethanol (31, 45, 29)
- isopropanol (45, 43, 29)
- chloroform (47, 83, 85)

Explosives Chemistry, Fire Debris
- methanol (31, 32, 29)
- ethanol (31, 45, 29)
- isopropanol (45, 43, 29)
- methyl ethyl ketone (43, 72, 29)
- nitromethane (30, 61,46)
- acetone (43, 58)
- toluene (91, 92, 65)

8.3 HS-NPD Testmix

The peaks of both cyanide and acetonitrile (or the alternate positive control) should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. Peak areas should compare favorably to previous analyses of the performance standard. The retention times of each component should be similar to previous analyses (unless GC maintenance has been performed, such as column clipping or replacement).

8.4 HS-FID Testmix

The peaks of all four analytes should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. Peak areas should compare favorably to previous analyses of the performance standard. The retention times of each component should be similar to previous analyses (unless GC maintenance has been performed, such as column clipping or replacement).

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

12 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. Acid liberates hydrogen cyanide gas and care must be taken to isolate acid solutions from cyanide sources. Refer to the *FBI Laboratory Safety Manual* for the proper handling and disposal of all chemicals. Personal protective equipment should be used when handling any chemical and when performing any type of analysis. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

13 References

Manufacturer(s)'s Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual.*

"Gas Chromatograph General Maintenance Protocol" (Inst 002) *Instrument Operation and Systems Support SOP Manual.*

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual.*

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
7	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Updated Section 3 s. Changed to 'appropriate instrument support personnel' in Sections 6.1 b & f and 6.2 b & g. Updated vacuum to '6 x 10^{-5} torr' in 6.1 c. Updated Section 8.1 c & d to account for instrument variation and maintenance. Heading updated in Section 5. Updated 'Instrument Operation and Systems Support' in Section 13 and header.
8	04/01/21	Section 1- added FID, revised to new disciplines/sub-disciplines. Section 2- incorporated FID as another detector option. Section 3- minor edits, incorporated FID and materials, added MassHunter Software, removed Gerstel specifics. Section 4- removed Reagent Log throughout; updated expiration information for consistency. Section 4.1- added cyanide as an example. Sections 4.2, 7.2, 8.2- added "Fire Debris" Section 4.3- modified title for simplicity; updated acetonitrile specifics (was 0.04%, 50uL to a 20mL headspace vial). Added sections 4.4, 6.3, 7.4, and 8.4 Section 5- changed title from "Sampling or Sample Selection". Section 6- changed title from "Procedures"; inserted specific sections to reference throughout (rather than "of this protocol", for example). Edited throughout for clarity, brevity. Section 6.2- reversed steps c) and d); added last sentence to step e). Reversed order of sections 8.1 and 8.2 (did not mark with change indicators since actual content did not change). Added retention time to section 8.3. Removed one reference.

Approval

Redacted - Signatures on File

General Chemistry Technical Leader:	Date: 03/31/2021
Toxicology Technical Leader:	Date: 03/31/2021
Explosives (Chemistry) Technical Leader:	Date: 03/31/2021
IOSS Manager:	Date: 03/31/2021
Fire Debris Technical Leader:	Date: 03/31/2021
Chemistry Unit Chief:	Date: 03/31/2021
Explosives Unit Chief	Date: 03/31/2021